

UNCLASSIFIED

RDT&E BUDGET ITEM JUSTIFICATION SHEET (R-2 Exhibit)

DATE

FEBRUARY 2000

BUDGET ACTIVITY

1 - Basic Research

PE NUMBER AND TITLE

0601384BP CHEMICAL/BIOLOGICAL DEFENSE (BASIC RESEARCH)

| COST (In Thousands) | FY 1999 Actual | FY 2000 Estimate | FY 2001 Estimate | FY 2002 Estimate | FY 2003 Estimate | FY 2004 Estimate | FY 2005 Estimate | Cost to Complete | Total Cost |
|--|-------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|------------|
| Total Program Element (PE) Cost | 28505 | 44040 | 33197 | 30990 | 30004 | 30973 | 31969 | Continuing | Continuing |
| CB1 CHEMICAL/BIOLOGICAL DEFENSE (Basic Research) | 5662 | 8541 | 2452 | 3565 | 3279 | 3468 | 3585 | Continuing | Continuing |
| TB1 MEDICAL BIOLOGICAL DEFENSE (Basic Research) | 15108 | 27502 | 20753 | 17777 | 18428 | 18763 | 19382 | Continuing | Continuing |
| TC1 MEDICAL CHEMICAL DEFENSE (Basic Research) | 7735 | 7997 | 9992 | 9648 | 8297 | 8742 | 9002 | Continuing | Continuing |

A. Mission Description and Budget Item Justification:

This program element (PE) funds the Joint Service core research program for chemical and biological (CB) defense (medical and non-medical). The basic research program aims to improve the operational performance of present and future Department of Defense (DoD) components by expanding knowledge in militarily relevant fields for CB defense. Moreover, basic research supports a Joint force concept of a lethal, integrated, supportable, highly mobile force with enhanced performance by the individual soldier, sailor, airman, or marine. Specifically, the program promotes theoretical and experimental research in the chemical, biological, and medical sciences. Research areas are determined and prioritized to meet Joint Service needs as stated in mission area analyses and Joint operations requirements, and to take advantage of scientific opportunities. Basic research is executed by academia, including Historically Black Colleges and Universities and Minority Institutions (HBCU/MIs), industry, and government research laboratories. Funds directed to these laboratories and research organizations capitalize on scientific talent, specialized and uniquely engineered facilities, and technological breakthroughs. The work in this program element is consistent with the Joint Service Nuclear, Biological, and Chemical (NBC) Defense Research, Development, and Acquisition (RDA) Plan. Management of funding resources leads to expeditious transition of the resulting knowledge and technology to the applied research (PE 0602384BP) and advanced technology development (PE 0603384BP) activities. This project also covers the conduct of basic research efforts in the areas of real-time sensing and diagnosis and immediate biological countermeasures. The projects in this PE include basic research efforts directed toward providing fundamental knowledge for the solution of military problems and therefore are correctly placed in Budget Activity 1.

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| B. <u>Program Change Summary:</u> | <u>FY 1999</u> | <u>FY 2000</u> | <u>FY 2001</u> |
|---|-----------------------|-----------------------|-----------------------|
| Previous President's Budget (FY 2000/FY 2001) | 29500 | 31386 | 31332 |
| Appropriated Value | 28697 | 44886 | |
| Adjustment to Appropriated Value | | | |
| a. Congressional General Reductions | | | |
| b. SBIR/STTR | -477 | | |
| c. Omnibus or Other Above Threshold Reductions | 565 | -89 | |
| d. Below Threshold Reprogramming | -280 | -287 | |
| e. Rescissions | | -470 | |
| Adjustments to Budget Years Since FY 2000/2001 PRES BUD | | | 1865 |
| Current Budget Submit (FY2001/PRES BUD) | 28505 | 44040 | 33197 |

Change Summary Explanation:

Funding: FY00 - Congressional Adjustments - CB1 (6500) for chemical biological detectors; chemical biological detector programs; research for optical devices for standoff chemical biological detection. TB1 (7000) for medical biological research.

Schedule:

Technical:

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BUDGET ACTIVITY

1 - Basic Research

PE NUMBER AND TITLE

0601384BP CHEMICAL/BIOLOGICAL DEFENSE (BASIC RESEARCH)

PROJECT

CB1

| COST (In Thousands) | FY 1999 | FY 2000 | FY 2001 | FY 2002 | FY 2003 | FY 2004 | FY 2005 | Cost to | Total Cost |
|--|---------|----------|----------|----------|----------|----------|----------|------------|------------|
| | Actual | Estimate | Estimate | Estimate | Estimate | Estimate | Estimate | Complete | |
| CB1 CHEMICAL/BIOLOGICAL DEFENSE (Basic Research) | 5662 | 8541 | 2452 | 3565 | 3279 | 3468 | 3585 | Continuing | Continuing |

A. Mission Description and Budget Item Justification:

Project CB1 CHEMICAL/BIOLOGICAL DEFENSE (Basic Research): This project funds basic research in chemistry, physics, mathematics and life sciences, fundamental information in support of new and improved detection technologies for biological agents and toxins; new and improved detection technologies for chemical threat agents; advanced concepts in individual and collective protection, new concepts in decontamination and information on the chemistry and toxicology of threat agents and related compounds.

FY 1999 Accomplishments:

- 920 Biosensors - Performed sequencing of high affinity recognition elements and expanded the list of target bioagents. Synthesized fluorescent detection complexes and began integration with epoxy chips. Demonstrated significant increase in sensitivity of an immunodetection method using dendrimer bound antibody.
- 1130 Aerosol Science - Assembled and tested a laboratory technology to allow visualization of changes in growing bacterial cultures as a rapid detection method for bio-active threats. Completed scattering model theorem and mathematical simplification to allow it to run in reasonable times on small computers for stand off and point detection of biological particles in air.
- 242 Chemistry and Toxicology of Bioactive Compounds - Tested specific or hepatocyte-dependent cytotoxicity on liver cells to determine if biotransformation of the test cell results in a product capable of inducing a cytotoxic effect in a particular target organ cell line. Began to establish binding selectivity with agent simulants of molecular imprinting detection mechanism. Initiated studies of potential protective overcoatings for molecular imprinting detection mechanism. Initiated study of a percarbonate based reactive decontaminant formulation.
- 3370 Man-Portable Thin-Film Detection Technology - Initiated studies on the control of variability in film quality, and stability through the use of silane linkages onto piezoelectric materials. Explored the use of "shape" selective surfaces with attachment of biomolecules and the mechanism for interaction on semiconductor metal oxide (SMO) sensing elements.

Total 5662

Project CB1

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1 - Basic Research

PE NUMBER AND TITLE

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PROJECT

CB1**FY 2000 Planned Program:**

- 836 Biosensors - Deliver purified oligomer recognition elements for the detection of Bacillus anthracis and Yersinia pestis. Continue conjugate synthesis and integration of specific fluorescent polymer/binding agent complexes. Continue synthesis of antibody/dendrimer tag complexes and begin work on the demonstration of separation/identification of dendrimer bound antibody/antigen couples via capillary electrophoresis.
- 1031 Aerosol Science - Based on refined scattering model theorem, begin design and fabrication of an instrument to measure the backscatter and image particles, thereby confirming the theorem's projections.
- 250 Chemistry and Toxicology of Bioactive Compounds - Complete project on cytotoxicity screening methods and transition the work to routine use throughout the toxicology program. Make a selection of the coating technology to be used in the molecular imprinting technique for the Individual Passive Chemical Agent Detector project. Continue rate studies on the percarbonate based decontaminant formulation to include work with surety materials. Expand peroxide activators to other technologies with promise for greater percent hydrogen ion (pH) range efficacy. Begin project to create a filtration performance model based upon an understanding of adsorption equilibria and rate processes; begin with development of database of adsorption equilibrium measurements.
- 6300 Man Portable Thin Film Technology - Continue development and refinement of SMO thin film technology with controlled architecture to detect chemical agents (e.g. nerve, blister, blood) and interferent species (e.g. volatile hydrocarbons, water, and other battlefield interferents). Development would optimize films for both point and cumulative exposure detection applications. Conduct laboratory testing to optimize the sensitivity, selectivity, and stability of SMO sensor elements and arrays as a function of gas environments.
- 124 SBIR/STTR.

Total 8541

FY 2001 Planned Program:

- 971 Biosensors - Perform DNA sequencing of the recognition elements to anthrax spores, cholera toxin and Staphylococcal enterotoxin B. Complete conjugate synthesis and chip integration of specific DNA/fluorescent polymer conjugates. Demonstrate separation/identification of dendrimer bound antibody/antigen couples via capillary electrophoresis.
- 1192 Aerosol Science - Complete confirmation of the scattering model theorem by demonstrating imaging of biological cluster particles. Transition the technology to the applied research program for further development.

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1 - Basic Research

PE NUMBER AND TITLE

0601384BP CHEMICAL/BIOLOGICAL DEFENSE (BASIC RESEARCH)

PROJECT

CB1**FY 2001 Planned Program (Cont):**

- 289 Chemistry and Toxicology of Bioactive Compounds - Continue studies of the percarbonate based decontaminant formulations by determining reaction product distributions and correlate equilibrium concentrations with solvent properties. Complete measurement of requisite adsorption rate data and begin development of a continuous adsorption model for filter performance. Begin new project to determine how novel organophosphorus compounds may achieve delayed toxic effects without inducing initial common symptoms.

Total 2452

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BUDGET ACTIVITY

1 - Basic Research

PE NUMBER AND TITLE

0601384BP CHEMICAL/BIOLOGICAL DEFENSE (BASIC RESEARCH)

PROJECT

TB1

| COST (In Thousands) | FY 1999 | FY 2000 | FY 2001 | FY 2002 | FY 2003 | FY 2004 | FY 2005 | Cost to | Total Cost |
|---|---------|----------|----------|----------|----------|----------|----------|------------|------------|
| | Actual | Estimate | Estimate | Estimate | Estimate | Estimate | Estimate | Complete | |
| TB1 MEDICAL BIOLOGICAL DEFENSE (Basic Research) | 15108 | 27502 | 20753 | 17777 | 18428 | 18763 | 19382 | Continuing | Continuing |

A. Mission Description and Budget Item Justification:

Project TB1 MEDICAL BIOLOGICAL DEFENSE (Basic Research): This project funds basic research, i.e., pre-Milestone (MS) 0, on the development of vaccines and therapeutic drugs to provide effective medical defense against validated biological threat agents including bacteria, toxins, viruses, and other agents of biological origin. This project also funds basic research employing biotechnology to rapidly identify, diagnose, prevent, and treat disease due to exposure to biological threat agents. Broad categories for this project include countermeasures (vaccines and therapeutics) to bacteria, toxins, and viruses; diagnostics; and broad-spectrum generic medical countermeasures (not agent specific). A subset activity underlying each category is the development of an understanding of the pathogenesis (mechanisms of disease) of these agents to include an understanding of functional genomics of threat agents. Acquiring complete genetic information of validated and novel threat agents provides a strong foundation for development of future medical countermeasures and rapid diagnostics. The broad categories may occasionally address more than a single agent group (i.e., bacterial/viral countermeasures reflects a focus on both groups of agents).

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1 - Basic Research

PE NUMBER AND TITLE

0601384BP CHEMICAL/BIOLOGICAL DEFENSE (BASIC RESEARCH)

PROJECT

TB1**FY 1999 Accomplishments:**

- 2524 Bacterial Countermeasures - Identified and characterized expression systems for virulence factors cloned from bacteria and rickettsia (glanders and typhus organisms), and performed isoelectric point analyses of the three protein components of anthrax toxins to better understand genetic differences among isolates; characterized antigenic relationships among numerous strains of Burkholderia mallei (glanders) and evaluated the sensitivity and specificity of an immunologic assay (ELISA) for use in vaccine development studies for glanders.
- 1125 Bacterial Countermeasures - Identified and evaluated the biological activity of several virulence factor proteins of Yersinia pestis for their role in pathogenesis.
- 1279 Bacterial Countermeasures - Investigated the capability of various adjuvants to enhance mucosal immunity to Brucellae for a potential novel vaccine approach to this threat agent; evaluated the expression system for multivalent Brucella vaccine to obtain cross-protection against various pathogenic strains.
- 1372 Toxin Countermeasures - Determined mechanisms of action of Staphylococcal enterotoxin-induced shock and evaluated a variety of inhibitors of these mechanisms.
- 2552 Toxin Countermeasures - Evaluated potential drugs for post-exposure therapies against ricin toxin using in vitro model system; identified potential inhibitors of the enzymatic activities of botulinum neurotoxin for further study.
- 1377 Diagnostics - Identified and explored new technologies and new antigens and gene markers for medical diagnostics.
- 680 Generic Medical Countermeasures - Evaluated generic countermeasures, such as broad-spectrum antitoxin and antiviral drugs, immunomodulators, and other therapeutics that are not agent-specific. Expanded basic research efforts to understand agent pathogenesis and the immunology of protection against threat agents.
- 791 Viral Countermeasures - Evaluated a broad variety of antiviral compounds for activity against filoviruses using in vitro (non-animal) model systems; further characterized mechanisms of immunity for Venezuelan, eastern, and western equine encephalitis viruses.
- 1511 Viral Countermeasures - Identified molecular targets for therapeutic compounds for protection against filoviruses and orthopox viruses.
- 1751 Bacterial/Viral Countermeasures - Continued full genome sequencing of various biological threat agents and initiated a gene bank (extensive database) search and analysis for a general virulence factor sequence.
- 146 Bacterial/Viral/Toxin Countermeasures - Identified genes coding for antigens from biological threat agent bacteria, viruses and toxins for their suitability for incorporation into multi-agent vaccine technological approaches, to include the virus replicon vaccine vector and naked DNA combination vaccines.

Total 15108

Project TB1

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1 - Basic Research

PE NUMBER AND TITLE

0601384BP CHEMICAL/BIOLOGICAL DEFENSE (BASIC RESEARCH)

PROJECT

TB1**FY 2000 Planned Program:**

- 6619 Diagnostics (Medical Biological Defense Research Program) - Generate full-length genetic sequence information of emerging and genetically engineered agents and re-evaluate classical biological warfare and bioterrorism threat agents; perform structural studies for toxins and critical enzymes using X-ray crystallography and other cutting-edge analytical methodologies.
- 5109 Viral Countermeasures - Assess modes of therapeutic protection to filoviruses and orthopox viruses provided by executing drug screening of pharmacological compounds provided by industry; explore novel vaccine strategies for these agents and investigate genetically engineered vaccine candidates (attenuating mutations) for remaining strains of eastern equine encephalitis viruses.
- 1130 Diagnostics - Access biotechnological innovations (i.e., Tech Watch) to provide rapid diagnostic capabilities leading to enhanced warfighter care. Conduct research using new technologies for military application.
- 3515 Bacterial Countermeasures - Evaluate expression system for multivalent Brucella vaccine; continue studying pathogenesis, host immune responses, virulence factors, strain diversity, molecular pathogenesis and correlates of immunity for organisms responsible for plague (Yersinia pestis), glanders (Burkholderia mallei), and anthrax (Bacillus anthracis).
- 4343 Toxin Countermeasures - Identify molecular biology and target mechanisms of action of botulinum toxin and Staphylococcal enterotoxins for exploitation in investigating therapeutic approaches to toxin exposure.
- 590 Generic Medical Countermeasures - Discover and design potential models/systems with emphasis on computer modeling (rational drug design) and in vitro systems for replacement of animal models in biomedical research.
- 1721 Generic Medical Countermeasures - Evaluate generic medical countermeasures, such as broad-spectrum antitoxin and antiviral drugs, immunomodulators, and other therapeutics that are not agent-specific. Incorporate latest scientific advances in immunology into the basic research efforts to understand agent pathogenesis and the immunology of protection against threat agents. Continue investigation of surrogate markers of efficacy for current and future vaccines and therapies.
- 1453 Bacterial/Viral Countermeasures - Obtain genetic sequencing data from a panel of validated threat agents; establish genetic sequences into a database; evaluate sequence data for the potential for genetic engineering and genetic modification of the pathogens; determine genetic fingerprints (genetic identifiers) of various isolates of the organism responsible for plague (Yersinia pestis).
- 1494 Bacterial/Viral Countermeasures - Study host cellular and sub-cellular responses to BW threat agent(s) (anthrax, filoviruses) exposure utilizing microassay technologies and focusing on gene regulation, to identify likely molecular targets for intervention for developing "next generation" (i.e., beyond present day) novel therapeutic strategies for exposures to various biological warfare agents.

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PE NUMBER AND TITLE

PROJECT

1 - Basic Research**0601384BP CHEMICAL/BIOLOGICAL DEFENSE (BASIC RESEARCH) TB1****FY 2000 Planned Program (Cont):**

- 1130 Bacterial/Toxin Countermeasures - Refine aerosol exposure animal models (multiple species) required to address FDA regulatory requirements for botulinum toxin, ricin, Staphylococcal enterotoxin B (SEB), and glanders.
- 398 SBIR/STTR.

Total 27502

FY 2001 Planned Program:

- 4533 Bacterial/Viral Countermeasures - Continue studying host cellular and sub-cellular responses to BW threat agent(s) (anthrax, filoviruses) exposure utilizing microassay technologies and focusing on gene regulation, to identify likely molecular targets for intervention; evaluate possible generic intervention points in agent-induced pathophysiology.
- 1712 Bacterial/Viral Countermeasures - Assess broad spectrum therapeutic strategies for exposures to multiple biological warfare threat agents. These strategies will focus on intervention in disease pathogenesis at the molecular level, and identify common host cellular targets for the pathogenic response.
- 3875 Viral Countermeasures - Evaluate results from previous assessment of new compounds that provide protection against filoviruses and orthopox viruses to determine potential new pre- and/or post-exposure therapeutic compounds for further study; assess new genetic technologies for applicability to vaccines for the viral threat agents.
- 596 Generic Medical Countermeasures - Design potential models/systems with emphasis on computer modeling, and in vitro systems for replacement of animal models in biomedical research.
- 2302 Bacterial/Viral/Toxin Countermeasures - Identify generic medical countermeasures against threat agents in suitable model systems; define likely targets in agent pathogenesis and host immune response; establish mechanisms for determining antibiotic resistance; and characterize promising surrogate markers of efficacy for selected vaccines and therapies for further exploratory development research.
- 3976 Bacterial Countermeasures - Investigate pathogenesis (somatic and molecular), host immune responses, virulence factors, strain diversities, and correlates of immunity for the causative agents of plague (Y. pestis), glanders (B. mallei) and anthrax (B. anthracis).
- 3759 Toxin Countermeasures - Identify sites of molecular action and mechanisms of intervention for therapies for botulinum toxin and Staphylococcal enterotoxin (SE) threats; develop models for therapeutic intervention for therapies for botulinum toxin and SE threats.

Total 20753

Project TB1

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BUDGET ACTIVITY

1 - Basic Research

PE NUMBER AND TITLE

0601384BP CHEMICAL/BIOLOGICAL DEFENSE (BASIC RESEARCH)

PROJECT

TC1

| COST (In Thousands) | FY 1999 | FY 2000 | FY 2001 | FY 2002 | FY 2003 | FY 2004 | FY 2005 | Cost to | Total Cost |
|---|---------|----------|----------|----------|----------|----------|----------|------------|------------|
| | Actual | Estimate | Estimate | Estimate | Estimate | Estimate | Estimate | Complete | |
| TC1 MEDICAL CHEMICAL DEFENSE (Basic Research) | 7735 | 7997 | 9992 | 9648 | 8297 | 8742 | 9002 | Continuing | Continuing |

A. Mission Description and Budget Item Justification:

Project TC1 MEDICAL CHEMICAL DEFENSE (Basic Research): This project emphasizes understanding of the basic action mechanisms of nerve, blister (vesicating), blood, and respiratory agents. Basic studies are performed to delineate mechanisms and site of action of identified and emerging chemical threats to generate required information for initial design and synthesis of medical countermeasures. In addition, these studies are further designed to maintain and extend a science base.

FY 1999 Accomplishments:

- 1331 Pretreatments - Using knowledge gained from structural studies, initiated development, using recombinant technology along with site-directed mutagenesis, to develop next generation protection for acute nerve agent poisoning.
- 135 Pretreatments - Synthesized reactive components for use in development of a protective skin barrier cream.
- 3685 Therapeutics - Screened 707 compounds from several chemical classes for viable blister agent post-exposure therapeutic properties.
- 439 Therapeutics - Evaluated novel temporary wound dressing for skin exposed to sulfur mustard (HD). Drafted approaches for use of accelerators of healing for HD-induced wounds.
- 1946 Therapeutics - Established a database of studies relevant to the underlying effects of low-level exposure(s) to CW agents.
- 199 Therapeutics - Evaluated novel drugs as countermeasures against nerve agents to prevent the brain damage and behavioral incapacitation that occurs following nerve agent-induced seizures.

Total 7735

FY 2000 Planned Program:

- 1008 Pretreatments - Develop appropriate knowledge for molecular modeling and site-directed mutagenesis to optimize next generation antidotes to nerve agent poisoning.

Project TC1

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PROJECT

TC1**FY 2000 Planned Program (Cont):**

- 1845 Therapeutics - Explore potential for new technologies to intervene or serve as biomarkers in the mustard injury cascade.
- 4101 Therapeutics - Expand database of studies relevant to effects of low-level exposure to chemical warfare agents; determine mechanism of nerve agent toxicity at low doses; identify data gaps. Begin studies to address long-term effects of acute and chronic low-level exposure to chemical warfare agents.
- 927 Therapeutics - Screen antidotes and countermeasures for efficacy against novel threat agents.
- 116 SBIR/STTR.

Total 7997

FY 2001 Planned Program:

- 4546 Diagnostics - Develop highly sensitive, forward deployable assay techniques to determine exposure to low levels of CW agents and subsequent physiological and toxicological effects.
- 2191 Pretreatments - Based on current research strategies, identify new candidate compounds or compound families with potential as pretreatments for vesicant injury.
- 543 Pretreatments - Using knowledge gained from studies in molecular modeling and site-directed mutagenesis, develop next generation antidotes.
- 2146 Therapeutics - Determine mechanism of action of percutaneous exposure to novel threat agents to explain the variation in response to current nerve agent countermeasures.
- 566 Therapeutics - Initiate research efforts to clearly define the optimal hypochlorite concentration for use in decontaminating chemical agent exposed skin and agent contaminated wounds.

Total 9992